QuikChange Site Directed Mutagenesis

Primer Design Guidelines

- Both of the mutagenic primers must contain the desired mutation and anneal to the same sequence on opposite strands of the plasmid.
- Primers should be between 25 and 45 bases in length, with a melting temperature (Tm) of ≥78°C. Primers longer than 45 bases may be used, but using longer primers increases the likelihood of secondary structure formation, which may affect the efficiency of the mutagenesis reaction.
- The following formula is commonly used for estimating the *T*m of primers:

  $T_m = 81.5 + 0.41(\%GC) - (675/N) - \%\ mismatch$

  - *N* is the primer length in bases
  - values for **%GC** and **% mismatch** are whole numbers
- For calculating *T*m for primers intended to introduce insertions or deletions, use this modified version of the above formula:

  $T_m = 81.5 + 0.41(\%GC) - (675/N)$

  where N does not include the bases which are being inserted or deleted.
- The desired mutation (deletion or insertion) should be in the middle of the primer with ~10–15 bases of correct sequence on both sides.
- The primers optimally should have a minimum GC content of 40% and should terminate in one or more C or G bases.

PCR Reaction

- Use 125 ng of each primer. To convert nanograms to picomoles of oligo, use the following equation:

  X pmoles of oligo = (ng of oligo)/(330 x #of bases in oligo) x 1000

  For example, for 125 ng of a 25-mer:
  (125 ng of oligo)/(330 x 25 bases) x 1000 = 15 pmole

- Use standard Phusion PCR protocol with following modifications:
  (i) elongation time ~1 minute for 1 kb
  (ii) 12 cycles
  (iii) Annealing temperature 60°C
  It usually works well to try different template DNA concentrations (e.g. 5, 10, 20 and 50 ng).
  As a control, prepare a reaction without Phusion (should give no colonies)

DpnI digestion

1 µl DpnI/PCR reaction
Incubate 60 min at 37°C

***E. coli* transformation**

According to a standard protocol, with 10 µl PCR reaction

Protocol generously provided by the lab
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